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EFFECT OF BENZYLADENINE ON MICROPROPAGATION OF BANANA SHOOTS TIP

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ABSTRACT

The effect of benzyl adenine (BA) at 0, 2, 4, 6 and 8 mg l^{-1} on micropropagation of banana shoot tips was studied. This study also included the morphological responses of banana shoot tips especially with 0 & 6 mg l^{-1} of BA treatments in relation to some biochemical compositions (total soluble phenols, free amino acids, total soluble sugars, chlorophyll a, b and carotenoids). Growth in 6 mg l^{-1} of BA resulted in increase in the most morphological parameters compared to the rest treatments. The results showed that 6 mg l^{-1} of BA treatment significantly increased fresh and dry weights, number of shootlets, shootlet and root length and number of leaves and roots/plantlet as compared without BA. Accumulation of total soluble sugars, free amino acids and chlorophylls was enhanced by 6 mg l^{-1} of BA while the reverse was true with the rest biochemical compositions (total soluble phenols and carotenoids). The biochemical status and BA treatment at 6 mg l^{-1} during micropropagation of shoot tips in banana may be important for the development and optimization of strategies for large scale propagation and germplasm conservation.

Keywords: Banana, Micropropagation, Shoot tip, Benzyladenine (BA=BAP)

INTRODUCTION

Banana (*Musa accuminata* L.) is a monocotyledon plant. Banana gives a huge number of individuals all through the tropics and subtropics with staple sustenance and records for a standout amongst the most broadly sent out organic products on the planet. They are goliath herbs usually grow up to 3-5 m in stature, with no lignification or auxiliary thickening of stems. The beginning of the gathering is from South to East Asia, happen from India to Polynesia. The plants are circulated predominantly on edges of tropical rainforests **(Heslop-Harrison and Schwarzacher 2007)**. Banana is the fourth biggest expended sustenance trim on the world **(Vuylsteke 1989)**. It is a nutritious organic product rich in starch and a decent wellspring of vitamins. Banana contains low protein level and can be utilized to deliver huge measure of recombinant proteins (i.e. immunizations) **(Arvanitoyannis et al 2008)**. Banana is utilized as a decent wellspring of drinks, fermentable sugars, aroma, rope, cordage, festoons, protect, smoking material, and various formal and religious uses **(Nelson et al 2006)**.

Banana plants are generally engendered vegetatively by suckers which develop from parallel buds beginning from corms, and suckers are isolated for creation of individual plants. In a few cases, finish or splitted corms with one or a few buds are utilized **(Vuylsteke et al 1993)**. Just 5 to 10 suckers can be acquired from a plant for every year in traditional strategy. Moreover, banana creation occasionally turns out to be genuinely influenced by various illnesses **(Rahman et al 2004)**. Accordingly, banana efficiency diminishes, and the yield turns out to be exceptionally poor.

To limit the previously mentioned issues, micropropagation could be an option for engendering of planting materials for banana. Micropropagation has turned into a basic piece of the banana production overall now as engendering by tissue culture from disease free mother plants. Tissue culture has many advantages of interest as the plant-

ing material don't convey foundational diseases, enthusiastic and are more uniform in their development, along these lines making the generation hones less and demanding prompting high efficiency. In this strategy, over a million of plant can be developed from a little or even a minuscule bit of plant tissue inside a year **(Mantell et al 1985)**. In addition, the shoot duplication cycle is short (75 days), each cycle bringing about an exponential increment in the quantity of shoots and plants augmentation can be proceeded during the time independent of the season **(Razdan 1993)**. Meristem culture offers a proficient technique for fast clonal proliferation, creation of infection free materials and germplasm safeguarding in plants **(Cronauer and Krikorian 1984; Hwang et al 2000 and Helloit et al 2002)**.

Therefore, this study designed to determine the effect of benzyl adenine (BA) on micropropagation of banana shoot tips and optimization of strategies for large scale propagation. In addition, some morphological parameters and biochemical changes were determined.

MATERIAL AND METHODS

Plant material

The suckers (70 – 80 old days) of *Musa acuminata* cv. Grand Nain AAA-group were collected from El-Rahman farm in Sadat city, Monufia Governorate, Egypt. The experiment was carried out in Plant Tissue Culture Lab of Agric. Botany Dept., Fac. of Agric. Ain Shams Univ., during the years of 2014–2016.

The selected suckers for the study were free from diseases. The suckers were washed thoroughly with running tap water after removing the leaves and the roots. The suckers were trimmed to a size of 5-6 cm in length and 2.5-3.5 cm width at the base. The pale white tissue block containing meristem and rhizomatous base were taken in a beaker. Surface sterilized with 70% ethanol for one minute, 0.1% mercuric chloride solution for 5 min. After that, the explants were rinsed three to four times with sterile distilled water 5 min. for each time. The surface sterilized meristem tissue blocks were then prepared under aseptic condition inside the laminar airflow cabinet by removal of outer tissue of meristem with the help of sterile scalpel until the length reached to 1.0 – 2.0 cm of its base. The individual meristematic dome of tissue was directly inoculated to the tissue culture jar containing MS medium **(Murashige and Skoog 1962)** supplemented with 30 g l^{-1} sucrose, 0.7% agar, 10 mg l^{1-} benzyl adenine (BA). The culture jars were incubated under dark for 60 days (swelling stage), then transferred under light condition for 16 h/day photoperiod at intensity of 3500 Lux from white fluorescent lamps at 25 °C ±2.

After initial establishment, shoot tips were subcultured on MS medium supplemented with 100 mg l^{-1} ascorbic acid, 100 mg l^{-1} myo-inositol, 0.4 mg l^{-1} thiamin HCl, 80 mg l^{-1} adenine sulphate, 100 mg l^{-1} tyrosine and 0, 2, 4, 6 and 8 mg l^{1-} BA for 6 times every 30 days (multiplication stage). When the shoots grown about 3-5 cm in length with 2-3 well developed leaves, they were separated from each other and transferred on MS medium supplemented with 2.5 g KH_2PO_4 for 30 days (shootlet thickness stage), finally transferred to freshly prepared free medium + activated charcoal 0.2% (root induction stage).

Growth parameters

Three replicates from each treatment were taken randomly after 180 days at the end of multiplication stage to record shootlets/jar. While the fresh weight (g), dry weight (g), plant height (cm), number of leaves/plantlet, number of roots/plantlet and root length (cm) were recorded after 240 days from swelling stage especially with 6 mg l^{-1} benzyl adenine (BA).

Biochemical analyses

Each one was determined in 0.5 g fresh weight of the plantlets. Determination of total soluble phenols, free amino acids, total soluble sugars, chlorophyll a, b and carotenoids were achieved at 30 days after root induction stage.

The total soluble phenols in the sample metabolic extracts were analyzed spectrophotometrically using a modification of the Folin–Ciocalteu colorimetric by the method of **Ozyigit et al (2007)** and expressed as mg/g fresh weight.

In the ethanol extract, free amino acids were determined calorimetrically by using ninhydrin solution according to **Swamy (2008)** and expressed as mg/g fresh weight.

Total soluble sugars (TSS) were extracted as per the strategy portrayed by **AOAC (2005)** and measured according to the method recorded by **Dubois et al (1956)** and expressed as mg/g fresh weight.

Chlorophylls (Chl a & b) and carotenoids (Car) were extracted and estimated as per the strategy portrayed by **Wellburn (1994)**. Formula and extinction coefficients were calculated using the following equations and expressed as mg/g fresh weight:

Chlorophyll a = 11.75 A662 – 2.350 A645
Chlorophyll b = 18.61 A645 – 3.960 A662
Carotenoids = 1000 A470 – 2.270 Chl a – 81.4 Chl b/227.

Statistical analysis

Data of treatments were arranged in complete randomized design **(Gomez and Gomez 1984)** and statistically analyzed for significance using analysis of variance (ANOVA) in statistix (8^{th} edition analytical software, USA) by **Steel et al (1997)**. Differences between means were contracted by Duncan's multiple range test $p<0.05$.

RESULTS AND DISCUSSION

Data illustrated in **Table (1)** showed the effect of different BA concentrations on the shootlets number from the banana suckers after 180 days at the end of multiplication stage. When sword suckers were developed on MS medium without any growth regulators (control), least morphogenetic response was observed during initiation of explant. While BA treatments were added to the medium, swelling was observed at the base of the shoot tip. Also, BA treatments increased shootlets number when compared to the control. Whereas, sprouting of photoactive green leaves were observed within two weeks of culture on MS medium enriched with BA, whilst reasonable appearance of shootlets was seen following three weeks **(Fig. 1)**. The fresh shoot tips produced two or three shoots within four weeks incubation when split longitudinally into two halves and in this manner inoculated in a similar medium for the second initiation. Followed by transferring the four split parts to solid MS medium containing BA treatments concentrations considered as first transfer then second, third and fourth up to six transfers. Upon sub culturing, these shoots after 4 weeks interval, each one produced an extra four to six shoots in the ensuing subculture. The average of multiplication ratio was even reached to several hundreds to thousands. The concentration 6 mg l^{-1} BA mostly gave the highest significant value of shootlets formation (901.98 shootlets) when compared with other concentrations.

Table 1. Effect of benzyladenine (BA) treatments on shootlets formation from banana suckers after 180 days after inoculation

BA (mg l^{-1})	0	2	4	6	8
Number of shootlets /explant	18.667e±3.05e	289.998d±3.51d	492.000b±5.0b	901.980a±5.507a	409.998c±3.51c

Means ± SD followed by different letters are significantly different at (P ≤ 0.05)

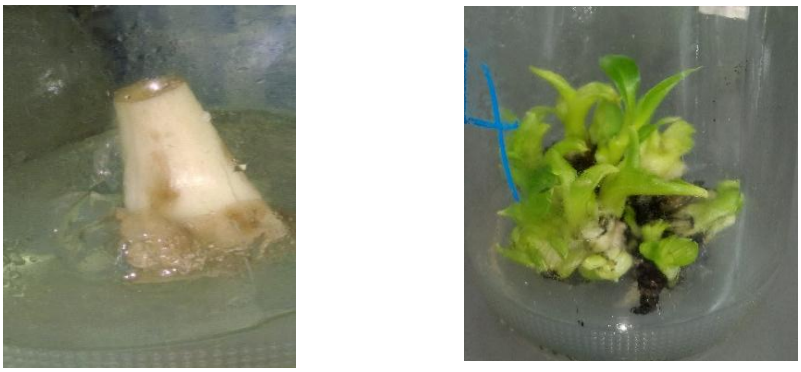

Fig. 1. Starting and sprouting of photoactive green leaves in response of BA treatments.

As for the effect of benzyl adenine on the growth parameters, data in **Table and Fig. (2)** revealed that the 6 mg l^{-1} BA treatment promoted and significantly increased most of the growth parameters except shoot length and number of leaves/plantlet. Previous studies reported that benzyl adenine (BA) is one of the synthetic cytokinins which assume tolerant part in the direction of different development forms in the plants **(Skoog et al 1967 and Ibrahim et al 2010)**. Cytokinins play an important role in shoots proliferation in tissue culture technique. **George (1993)** stated that the requirement for a cytokinin was sometimes noted for the promotion of adventitious shoots formation. For example, BA promoted axillary buds proliferation of *Castanea*. It was necessary to use BA to obtain multiple shoots. Level of cytokinins represents an important factor that affects the cultured explants. **Gangaprasad et al (2003)** found that multiplication and bud formation occurred with benzylaminopurine (BAP=BA). **Mishra and Sreenath (2003); Abdel Gayed et al (2006)** reported that frequency of shoot regeneration was positively correlated with the concentrations of BA. The results are in steady with that of **Choudhary et al (2014)** who reported that BA is the best for establishment of banana cultivar Robusta rather than combinations of various cytokinins and auxins. **Shibli et al (2001)** found that BA up to 1.0, 1.5 and 2.0 mg l^{-1} prompted an expansion in number of multiplied shoots and number of nodes per jar *in vitro* refined of *Solanum tuberosum* cv 'Spunta'. **Rahaman et al (2004)** recorded that the highest shoot number was at 4 mg l^{-1} BAP + 1.5 mg l^{-1} NAA (4.52 explant) at 30 days after inoculation. Also, **Rahman et al (2013)** tested the effect of different cytokines (BAP, 2iP and Kin) on the percentage of banana shoot proliferation, number of shoots/explant, the shoot length and number of leaves/plantlet. They found that BAP showed the best results compared to 2iP and Kin. **Ngomuo et al (2013)** concluded that 6 mg l^{-1} BA significantly enhancing the formation of banana shoots number and shoot fresh weight *in vitro*.

Table 2. Effect of 0 and 6 mgl^{-1} BA on banana suckers growth parameters after 240 days after inoculation

BA (mg l^{-1})	FWt. (g)	DWt. (g)	No. of shootlets	Shootlet length (cm)	No. of leaves/plantlet	No. of roots/plantlet	Root length (cm)
0	1.865±0.054b	0.063±0.006b	18.667e±3.05b	12.85±0.698a	5.42±1.003a	4.86±1.385b	14.18±2.231b
6	2.036±0.063a	0.089±0.008a	901.980±13.01a	13.9±0.793a	5.66±1.154a	7.66±1.527a	18.33±2.516a

Means ± SD followed by different letters are significantly different at (P ≤ 0.05)

Zero mg l^{-1} BA **Six mg l^{-1} BA**

Fig. 2. Effect of BA treatments on banana suckers growth parameters after 240 days.

Table 3. Effect of benzyl adenine (BA) at 0 and 6 mg l^{-1} on biochemical composition of banana plantlets after 240 days from starting

BA (mg l^{-1})	Total soluble phenols	Total soluble sugars	Free amino acids	Chl a	Chl b	Chl a+b	Cart.
	(mg/g F.Wt) ±SD						
0	0.487±0.011a	0.462±0.010b	0.893±0.030b	0.214±0.182a	0.154±0.020a	0.368±0.012a	0.120±0.010a
6	0.322±0.066b	0.889±0.016a	1.242±0.297a	0.236±0.020a	0.180±0.022a	0.415±0.009a	0.110±0.004a

Means ± SD followed by different letters are significantly different at ($P \leq 0.05$)

It is clear from data of **Table (3)** that benzyl adenine treatment led to decrease significantly the total soluble phenols when compared to control. On the other hand, the same treatment showed significant increasing in the total soluble sugars and free amino acids of the produced plantlets. On the contrary, there are no significant differences between benzyl adenine treatment and control on chlorophyll a, b and carotenoids concentrations. **Caers and Vendrig (1986)** reported that exogenous application of kinetin or other cytokinins promote chloroplast development. **Treharne et al (1970)** showed that foliar sprays of cytokinin stimulate photosynthesis and rubisco. The enhancement of photosynthesis and transport of photoassimilates may be the possible interpretation to the role of cytokinins in promotion growth. **Mucciarelli et al (2000)** reported that, many phenolic compounds probably act by raising or lowering the level of indole acetic acid (IAA) through enzymatic reactions. Thus, their studies suggested an involvement of benzoic acids in the control of auxin catabolism. Also, possible roles of these benzoic acids in cell metabolism and growth should be taken into account. Phenols were found to react with hydrogen peroxide produced during IAA degradation, thereby protecting the cell from its toxic effects. Rapid cell proliferation and active aerobic metabolism, which occur mainly in the presence of auxin and cytokinin, are often associated with the production of reactive oxygen species (ROS) **(Mittler 2002)**. Relatively large amounts of natural inhibitors of IAA oxidase have been reported to be present in meristematic and juvenile tissue, but not in normal mature differentiated cells until they are wounded **(George et al 2008)**.

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تأثير البنزيل ادينين علي الاكثار الدقيق للقمة الخضرية للموز

[189]

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الموجـــــــز

تم دراسة تأثير البنزيل ادينين بتركيزات 0، 2، 4، 6، 8 ملجم/لتر علي الاكثار الدقيق للقمة الخضرية للموز وكذلك أيضا علي الاستجابة المورفولوجية للقمم الخضرية لنبات الموز للمعاملات 0 ، 6 ملجم/لتر من البنزيل أدينين وعلاقتها ببعض المكونات الكيموحيويه (الفينولات الكليه الذائبة– الأحماض الأمينية الحرة – السكريات الكلية الذائبة – وكلوروفيلات أ ، ب والكاروتينويدات).

أدي استخدام البنزيل أدينين بتركيز 6 ملجم/لتر إلي زياده في بعض الخصائص المورفولوجية مقارنة مع المعاملات الأخري. أظهرت النتائج أن البنزيل أدينين بتركيز 6 ملجم/لتر زاد معنوياً الوزن الطازج والجاف وعدد الأفرع الخضرية وطول الفرع وعدد الأوراق والجذور للنبيتة و طول الجذر مقارنة بالمعاملة الخالية من البنزيل أدنين.

شجعت معاملة البنزيل أدينين بتركيز 6 ملجم/لتر تراكم السكريات الكلية الذائبة والأحماض الأمينية الحرة والكلوروفيلات و العكس كان حقيقي مع باقي المكونات البيوكيميائية (الفينولات الكلية الذائبه والكاروتينويدات). الحالة البيوكميائية والمعاملة بمادة البنزيل أدينين بتركيز 6 ملجم/لتر أثناء الإكثار الدقيق للقمة الخضرية في الموز ربما تكون مهمة لتطورها والاستراتيجية المثلي للإكثار علي مدي كبير وحفظ المادة الوراثية.

الكلمات الدالة: الموز، الإكثار الدقيق، القمة الخضرية، البنزيل ادينين

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